Table 3.

Preferred conditions for callus proliferation for various Taxus species. The ingredients in the basal media are listed in Table 2.

Species	Basal Medium (Table 2)	Growth Regulators*			
		Auxin		Cytokinin	
		Type	Conc (M)	Type	Conc(M)
T. brevifolia	F	P	5×10^{-6}	2iP	10^{-7}
	D	P	5×10^{-6}	BA	10^{-8}
T. canadensis	H	P	5×10^{-6}	K	10^{-7}
	D	P	5×10^{-6}	BA	10^{-8}
T. chinensis	D	P	5×10^{-6}	BA	10^{-8}
	A	N	5×10^{-6}	BA	10^{-8}
T. globosa	D	P	5×10^{-6}	BA	10^{-8}
T. floridana	D	P	5×10^{-6}	BA	10^{-8}
T. baccata	D	P	5×10^{-6}	BA	10^{-8}
T. cuspidata	D	P	5×10^{-6}	BA	10^{-8}
T. media	D	P	5×10^{-6}	BA	10^{-8}
T. wallichiana	D	P	5×10^{-6}	BA	10^{-8}

*Abbreviations: Picloram (P), Naphthalene acetic acid (N), Benzyladenine (BA), Dimethyl allylamino purine (2iP), Kinetin (K)

Table 4.

Typical growth characteristics of Taxus sp. suspension cultures

Species	Dry Weight Doubling Time	Fresh Weight Doubling Time	Dry Wt. Density	Fresh Wt. Density
T. brevifolia	2.0 days	3.5 days	20 g/L	400 g/L
T. baccata	2.0	6.0	15	220
T. chinensis	2.5	4.5	20	285
T. canadensis	nd*	8.5	13	260

*not yet determined

TABLE 7.

Effect of Standard GroLux light treatment on taxol and taxane content in 10-day old cultures of *Taxus chinensis* line K-1 cultivated in Medium A. Amounts shown are expressed as μg extracted from 20 ml of suspension. Cell growth was identical in both treatments (164 mg dry weight per flask).

	Light	Dark
Total taxol: cells and medium:	8.8 μg	3.13 μg
Extracellular taxol:	76.40%	56.20%
Total taxanes cells and medium:	61.55 μg	62.17 μg
Extracellular taxanes:	89%	84%

TABLE 8

Comparison of chitosan-glutamate treated to non-elicited suspensions of *Taxus chinensis* line K-1 after 15 days cultivation in medium C. Taxane levels reported are from cells and medium combined. % extra refers to the percentage of extracellular

	CONTROL			ELICITOR		
	Cell density		10.1 g/L	Cell density		14.2 gm/l
	Cell viability		70-80% viable	Cell viability		75-80% viable
Taxanes	% dry wt	mg/L	% Extra	% dry wt	mg/L	% Extra
Taxol	0.054	5.4	7.2	0.098	13.9	85.0
Baccatin III	0.057	5.8	69.9	0.055	7.8	76.6
7-Xylosyl-10-deacetyltaxol	0.040	4.0	63.0	0.048	6.9	77.0
10-deacetyltaxol	0.0004	0.4	71.1	0.0	1.0	75.3
Cephalomannine						
10-deacetylbaccatin III						
10-deacetyl-7-epitaxol	0.054	5.4	74.2	0.076	10.8	85.7
7-Epitaxol	0.009	0.9	74.6	0.009	1.3	86.2
Unknown Taxanes	0.203	20.5	79.7	0.240	34.1	90.2
Total Taxanes:	0.421	42.4		0.533	75.8	

Table10
Enhancement of Taxane Biosynthesis in *Taxus chinensis* cell line KS1A by Silver

Silver Compound	Dose (μM)	mg/L extracellular product**		
		Baccatin III	Taxol	Total Taxanes
Culture Medium only*		16	5	21
Silver thiosulfate	50	71	15	86
Silver phosphate	100	48	7	55
Silver benzoate	20	40	7	47
Silver sulfate	20	61	7	68
Toluenesulfonic acid silver salt	20	39	6	45
Silver chloride	10	22	18	40
Silver oxide	50	43	18	61
Silver acetate	10	52	10	62
Silver nitrate	20	63	6	69

* The culture medium was Medium N from Table 2, with the addition of the following growth regulators: 10 μM α-naphthaleneacetic acid, and 1 μM thidiazuron
** All samples were taken after 14 days of incubation.

Table 11.
Enhancement of Taxol and Taxane Biosynthesis by Silver in several *Taxus chinensis* cell lines. The titers represent levels measured in the whole broth, i.e., in the cells and in the extracellular medium.

Cell Culture	Silver[a] Concentration	Culture Medium	Duration (days)	Baccatin III mg/L	Taxol mg/L	Other Taxanes mg/L	Total Taxanes (mg/L)
SS6A-1224	0	I[b]	30	10	48	23	81
SS6A-1224	50 μM	I	30	172	86	126	384
SS122-13	0	II[c]	14	2	21	10	33
SS122-13	50 μM	II	14	12	103	60	173
SS122-42	0	II	14	3	80	26	109
SS122-42	50 μM	II	14	4	146	38	188

[a] Added as silver thiosulfate
[b] The culture medium is Medium N from Table 2, with the addition of the growth regulator, α-naphthaleneacetic acid at a concentration of 10 μM.
[c] The culture medium is Medium N from Table 2, with the addition of the growth regulator, α-naphthaleneacetic acid at a concentration of 10 μM and thidiazuron at a concentration of 1 μM.

Table 12
Enhancement of Taxol and Taxane Biosynthesis by Jasmonic acid and its methyl ester.Taxane titers were measured in the whole broth after 14 days of cultivation. The culture medium was Medium N from Table 2, with the additional presence of the growth regulator, α-naphthaleneacetic acid at a concentration of 10μM.

Cell Culture	Jasmonate Concentration	Baccatin III mg/L	Taxol mg/L	Other Taxanes mg/L	Total Taxanes (mg/L)
SS122-42	0	3	80	26	109
SS122-42	200 μM JMA	4	120	87	211
SS122-42	89 μM MJS	3	121	109	233
SS122-13	0	2	21	10	33
SS122-13	89 μM MJS	9	73	63	124

[a] JMA denotes the free acid, and MJS denotes methyl jasmonate

Table 13
Enhancement of Taxol and Taxane Biosynthesis by 3,4-methylenedioxynitrocinnamic acid (MDNA). Taxane levels were measured in the whole broth after 14 days of cultivation. The cell line used was *Taxus chinensis* SS122-42.

MDNA Concentration	Culture Medium[a]	Baccatin III mg/L	Taxol mg/L	Other Taxanes mg/L	Total Taxanes (mg/L)
0	I	3	80	26	109
50 μM	I	5	163	45	213
50 μM	II	34	311	89	434

[a] The culture medium I refers to Medium N from Table 2, with the additional presence of the growth regulator, α-naphthaleneacetic acid at a concentration of 10μM. The culture medium II is identical to Culture medium I, with the additional presence of 50 μM silver thiosulfate.

Table 14
Enhancement of Taxol and taxanes in cell cultures of *Taxus chinensis* using various combinations of enhancement agents. All taxane concentrations are expressed as whole broth titers (i.e., concentration in cells and medium combined), and values were obtained after 11 days of incubation.

Cell Culture	Culture Medium[a]	Baccatin mg/L	Taxol mg/L	Other Taxanes mg/L	Total Taxanes (mg/L)
SS64-412	I	41	464	101	606
SS64-561	II	590	182	388	1160
SS64-571	III	596	158	261	1015
SS124-77	IV	72	39	576	687
SS122-29	V	18	306	152	476
SS85-26	VI	586	100	416	1102

[a] The culture medium for all combinations was Medium N in Table 2. Culture Medium I contained, in addition to Medium N, 10 μM α-naphthaleneacetic acid (NAA), 3 μM thidiazuron (TDZ), 50 μM 3,4-methylenedioxynitrocinnamic acid (MDNA), 89 μM methyl jasmonate (MJS), and 50 μM silver thiosulfate (SLTS). Culture Medium II contained, in addition Medium N, 10 μM NAA, 1 μM TDZ, 50 μM MDNA, 89 μM MJS, 10 μM SLTS, and an additional 98.5 mg/L sodium phosphate (monobasic). Culture medium III contained, in addition to Medium N, 10 μM indolebutyric acid, 3 μM TDZ, 30 μM 3,4-methylenedioxycinnamic acid, 89 μM MJS, and 50 μM SLTS. Culture medium IV contained, in addition to Medium N, 10 μM NAA, 89 μM MJS, 100 μM SLTS, and 5 mM glutamine. Culture medium V contained, in addition to Medium N, 10 μM NAA, 89 μM MJS, and 50 μM SLTS. Culture medium VI contained, in addition to Medium N , 10 μM NAA, 1 μM TDZ, 50 μM MDNA, 18 μM MJS, 50 μM SLTS, and 5 mM glutamine.

Table 15
Enhancement of Taxane Production by Medium Exchange.

Cell Line	Culture Medium[a]	Type of Operation[b]	Duration (days)	Product[c]	Production Level[d] (mg/L)	Ave. Volumetric Productivity[e] (mg/L/day)
Paella	I	Batch	11	Taxol	185	13
Paella	I	Medium exchange	20	Taxol	265	17
SS29-3A5	II	Batch	14	Baccatin III	260	18
SS29-3A5	II	Medium exchange	28	Baccatin III	580	21
SS29-3A5	II	Batch	22	10-deacetyl-baccatin III	300	14
SS29-3A5	II	Medium exchange	28	10-deacetyl-baccatin III	400	14
SS45-146	III	Batch	11	Total Taxanes	700	64
SS45-146	III	Medium exchange	28	Total Taxanes	2500	89

[a] The culture medium for these culture conditions was Medium N in Table 2. Culture medium I included, in addition to Medium N, 10 μM α-naphthaleneacetic acid (NAA), 1 μM thidiazuron (TDZ), 50 μM 3,4-methylenedioxynitro-cinnamic acid (MDNA), 18 μM methyl jasmonate (MJS), and 10 μM silver thiosulfate (SLTS). Culture medium II included, in addition to Medium N, 10 μM NAA, 1 μM TDZ, 50 μM MDNA, 89 μM MJS,10 μM SLTS, and 5 mM glutamic acid (monopotassium salt). Culture medium III included, in addition to Medium N, 10 μM NAA, 2.5 μM zeatin, 30 μM MDNA, 89 μM MJS, and 50 μM SLTS.

[b] Repeated enhancement was achieved by medium exchange, as described in Example 14.

[c] The predominant product produced by a given cell line under the specified culture medium is listed; taxanes other than the predominant product were also produced in each case, except for cell line SS45-146, for which total taxane production is listed.

[d] The production levels for batch cultivation refer to extracellular concentrations, i.e., the amount of taxane measured in the extracellular medium divided by the volume of the extracellular medium. For repeated enhancement by medium exchange, the production level refers to the total amount of taxane measured in the extracelluar medium after each medium exchange, divided by the suspension volume.

[e] The average volumetric productivity is one indicator of biosynthetic capability; it is defined as the total product divided by the suspension volume, and futher divided by the duration of the incubation.

TABLE 16.b.

Details of fed-batch operation described in Table 16.a.

Feed solution	Composition	Feed rate (mL/L/day)	Start of feed (day)	Duration of feed (days)
F1	25% (weight/volume) (w/v) fructose, 25 mM glutamine, 50μM NAA, 250 μM SLTS, 89 μM MJS, 1.48 mM calcium chloride, 0.63 mM magnesium sulfate; 0.68 mM sodium phosphate (monobasic).	10	7	17
F2	F1, 75 mM α-phenylalanine, 25 mM β-phenylalanine	10	7	17
F3	25% (w/v) fructose, 150 mM α-phenylalanine, 25 mM β-phenylalanine	10	6	25
F4	50% (w/v) glucose, 5.92 mM calcium chloride, 2.52 mM magnesium sulfate, 2.72 mM sodium phosphate (monobasic), 500 μM SLTS, 10 μM TDZ, 100 μM NAA, 150 mM α-phenylalanine, 50 mM β-phenylalanine	5	9	22
F5	contained 50% (w/v) glucose, 100 μM NAA, 10 μM TDZ, 500 μM SLTS, 89 μM MJS, 0.68 mM sodium phosphate (monobasic), 50 mM α-phenylalanine	5	12	9